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The Effects of More Realistic Forms of Lead Heterogeneity in Soil on Uptake, Biomass and Root Response of Two *Brassica* Species

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Authors' contributions

This work was carried out by author GOSW. Author GOSW designed the study, wrote the protocol and the first draft of the manuscript, managed the literature searches, analysis of the study, performed the chemical analysis, greenhouse experiments and managed the experimental process. Author MHR supervised this work with the help of author EAJ on the plant aspect, author MHR read the first draft and author EAJ read the second draft.

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ABSTRACT

The spatial heterogeneity of soil constituents is known to have significant impacts on plant growth and plant uptake of nutrients and contaminants, yet studies have rarely used patterns of heterogeneity based on those found in the field. Heterogeneity refers to how lumpy materials are distributed in the soil, whilst homogeneity is the uniformity in the distribution of such materials. We identified patterns of lead contamination at historically polluted field sites and conducted pot trials using field–based parameters to determine the pattern of distribution of lead within the pots. We examined plant Pb uptake and growth in simulated low, medium and high heterogeneity environments as well as a control homogeneous treatment. We found a significant effect of Pb spatial heterogeneity on uptake and biomass of two *Brassica* species (*Brassica napus* and

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Brassica juncea), both candidate species for phytoremediation projects. Biomass was 4 to 5 fold lower in the high heterogeneity treatment and total plant Pb uptake as Pb mass in (µg) was 40 to 80% lower, compared to the homogeneous treatment. Plant lead concentration (mg/kg) increased by a factor of 2 with increasing heterogeneity. Peak uptake was observed in low and medium heterogeneity treatments of *B. napus* and *B. juncea* respectively. We also explored roots behaviour in the high heterogeneity treatment and found variation in root mass by 20 to 80% between concentric patches with significant ($P < 0.05$) differences between patches and species. High proportion of roots (40 to 50%) were proliferated in patches of lower Pb concentration. The tap root was a greater proportion of root in *B. napus*, which was absent in *B. juncea*. Results suggest that root morphology of this plant species might be a factor influencing the placement of roots in concentric patches and consequently the overall root response to Pb spatial heterogeneity. This is an indication that the root response could be realistic of that experienced by plants in field conditions. Generally result showed that spatial heterogeneity of Pb has a significant effect on plant growth and biomass. This study also demonstrated that the presence and extent of *in situ* heterogeneity of Pb in soil plays an important role in Pb uptake by plants. This work has implications for improving the phytoremediation of Pb contaminated land, phytomining, the reliability of risk assessment/models of human exposure to Pb and the quality of trace mineral content of agricultural produce.

Keywords: In situ heterogeneity; realistic simulation; Pb uptake; homogeneous; Brassica species; root proliferation; patch contrast.

1. INTRODUCTION

Lead pollution of soil especially in mining areas is a widespread and significant problem globally. There is an increasing amounts of Pb in soils of both arable and cultivated lands for various terrestrial ecosystems from anthropogenic sources [1]. An estimate of over half a million contaminated sites has been reported worldwide [2]. Lead contamination of soil can cause variety of environmental problems, including vegetation loss, ground water contamination and toxicity to plants, animals and humans especially children [3-5]. An estimated 5.9 to 11.7 million children worldwide are at risk from exposure to Pb via ingestion of Pb contaminated soil or inhalation of Pb solid phases [6-8]. Consumption of Pb contaminated food crops and vegetables is another potential route of exposure to human [9], [8]. Varied health effects are associated with such exposure. Plant uptake of Pb poses a potential health risk to both animals and humans. Interestingly, the same plant uptake mechanisms that pose potential risks to human from toxic heavy metals may also provide a possible solution to remediation of contaminated land by phytoremediation.

Increasing public concerns over the presence of heavy metal pollutants in the environment have led to a search for suitable technologies for clean-up of contaminated environments [10]. Phytoremediation has emerged as a low cost, low–maintenance, environmentally friendly and renewable technology for *in situ* stabilisation and removal of organic and inorganic contaminants from the environment, compared to existing decontamination methods [11].

The spatial pattern of contaminants in soil in which a plant is growing determines plant growth and contaminant uptake [12-14], and has been shown to have significant impact on the potential for crops to exceed contaminant safety limits or to be effective in phytoremediation projects. However, this has typically been established using simple binary models of heterogeneity and comparing such situations to homogeneous controls. Very little is known about how realistic patterns of heterogeneity, as found at contaminated sites (*in situ* heterogeneity), affects plant growth and contaminant uptake. Realistic heterogeneity is much more complex than binary heterogeneity.

Soil properties and constituents that affect plant growth are often heterogeneously distributed. Significant variation was found in nutrient resources at different scales around a single plant [15,16]. A strong effect of nutrient heterogeneity on plant biomass and acquisition of nutrient resources is well established [17-20,16,21,22,15,23], particularly when the heterogeneity is examined at scales similar or smaller in size than individual plants.

Heterogeneity of contaminant concentration has similar potential implications to those reported for nutrients, as both are common components of soil that can interact with plants. The study of heterogeneity in the distribution of trace metals (e.g. Cd and Zn) in the soil has received some attention in recent years. Earlier studies by [12], [24] and [14] using the simplistic binary ('hit and miss') heterogeneity in pot experiments showed significant differences in shoots and roots Cd and Zn concentrations compared to those grown in homogenized growth media with the same overall concentration of contaminant. The effects of heterogeneity may explain significant differences in plant uptake of contaminants between pot experiments in controlled (usually nominally homogeneous) environments and those in *in situ* studies [25].

Spatial heterogeneity of contaminants in soil refers to the pattern in which contaminants are distributed in the soil. A major factor that have been shown to impact significantly on plant uptake is the spatial heterogeneity of contaminants and scale of heterogeneity in relation to target receptors [12,14].

Understanding of spatial scales is relevant in the ecological studies of plants. [26,27] found that the impact of spatial heterogeneity of nutrient distributions relative to individual plant roots have significant effect on the performance of some plant species. [21,16,23,22] reported a strong effect of nutrient heterogeneity on plant biomass and acquisition of nutrient resources.

Some studies [12,13,28,29,14] have shown that the pattern and scale of Zn and Cd heterogeneity can have a significant effect on plant performance and uptake.

Much of the previous studies aimed at estimating plant uptake were based on pot experiments in hydroponics or homogeneously distributed trace metal medium [30-34] or a field site where the soil-plant system is peculiar to that site [35,36]. A major drawback of previous works on plant uptake is that spatial heterogeneity in contaminants distribution is overlooked. Studies using Zn and Cd [12-14] have shown significant differences between the checkerboard models used and homogeneous media, but these models are not characteristic of the original spatial patterns of contaminant heterogeneity experienced by plants in realistic field conditions. [14] showed that Zn heterogeneity seen in the field can be simulated in pot trials to assess its effect on plant uptake. That study [14] reported an extreme contrast between models used to map spatial distribution of trace metals in contaminated land investigations and the distribution of trace elements used in controlled studies to estimate plant uptake.

Differential root growth that might affect metal uptake has been shown in a number of plant species. Foraging traits, such as the localized root proliferation in patches of substrate with high metal concentrations may be important in enhancing heavy metal accumulation in hyperaccumulator species [24]. Some plants are able to forage for patchily distributed resources by positioning or proliferating leaves, roots or ramets when patches of higher quality or greater resource is available [37-39]. Previous studies e.g [40,20,27] showed that foraging responses such as root proliferation in response to local nutrient enrichment had been observed in many plant species and for some species, greater growth has been achieved in patchy habitats than in homogenous habitat. Patchy distribution of nutrients can influence plant performance as a result of altered resource acquisition, allocation patterns and changes in total biomass [26,41].

[42,43,24], observed a positive root proliferation in *Thlaspi caerulescens,* a Zn accumulator in response to substrate patches with high Zn concentration. [44,45] reported a non-foraging but positive response of *Pterris vittata* the arsenic hyperaccumulator plant, to spatial distribution of arsenic in soil.

In a recent study [46], *in situ* heterogeneity of Pb in soil was estimated over a range of scales from two site investigations using an *in situ* measurement technique with the specific sampling design proposed by [47]. The degree of heterogeneity was expressed as a heterogeneity factor (HF, explained below), where a homogeneous distribution would result in a HF value of 1 and heterogeneous distribution in a value of HF > 1 [46]. The use of heterogeneity factor to express *in situ* heterogeneity can be regarded as a new way to express heterogeneity information to aid interpretations in geochemical investigations. It has wider application in the diagnosis of the source of contamination and mode of deposition of contaminants using the HF values [46].

Heterogeneity factor values used in the pot trial described below reflect the scale of heterogeneity that can potentially affect the selected plant species in the volume of soil contained in the pot. *In situ* heterogeneity of Pb at the 0.02 m scale was chosen for the purpose of this experiment in relation to the size of root ball of *Brassica napus* and *Brassica juncea* which are food crops and potential Pb accumulators that could be used for phytoremediaton. Earlier work by [14] with zinc heterogeneity also used the 0.02 m scale that can be replicated within a pot trial. Similarly, [13] used a scale of 0.03 m with the simplistic chequer board models and showed that changes in heterogeneity of Cd can have a significant effect (+76%) on plant uptake.

In this study, we assessed the effects of realistic *in situ* heterogeneity of Pb in soil on plant uptake of Pb by a designed pot trial to mimic the range of *in situ* heterogeneity(in low, medium and high heterogeneity treatments) reported in earlier field investigation [46] and compared to a homogeneous treatment. We also examined the wider significance of the study in the estimation of human exposure to Pb and phytoremediation of Pb contaminated sites.

2. METHODS

2.1 Experimental Design

Four levels of *in situ* heterogeneity (Fig. 1) were simulated using excel computer models with a combination of Robust ANOVA- a visual basic programme developed based on a FORTRAN programme [48] and previous work (Analytical Method Committee [49]. These were designed to generate levels of heterogeneity that were similar to those that had been measured at field sites and in previous field studies [46].

The scale of heterogeneity used, the plant species selected, and the mean Pb concentration chosen, were based upon field investigation and conclusions of earlier pot trials in an unpublished thesis [50].

The actual spatial heterogeneity of contaminants can only be estimated by sampling at the field site, and it is practically impossible to recreate the exact *in situ* heterogeneity in pot trials. In view of this potential complexity, the models of heterogeneity (in Fig. 1) were designed to simulate as closely as practicably possible the *in situ* heterogeneity of Pb measured at this scale in field sites including some sites previously studied by other workers examined in [46]. The heterogeneity factor (HF) values (ranged from 1 {homogeneous} to 3.22 {highly heterogeneous}). The simulated heterogeneity factors (HF) were 1.00, 1.25, 2.00 and 3.19 for homogeneous, low, medium and high heterogeneity treatments respectively. An overall mean concentration of approximately 1000 mg/kg was maintained in all treatments (Figs. 1a-d) based on concentration range established from earlier pot experiment. The simulation is based on the $\log_e$-normal distribution of Pb concentration observed in the field sites, with increasing values of geometric standard deviation (GSD_{samp}) i.e. with the analytical uncertainty subtracted, and hence the increasing values of HF ($\exp^{GSDsamp}$). The central cell (C3) of all treatments was also consistently 1000 mg/kg Pb. This is to ensure that the different heterogeneity treatments did not differentially affect the early establishment of the seedlings.

2.2 Preparation of Growth Medium

The growth medium was a mixture of silver sand of grain size 0.063 - 0.2 mm and compost in the proportion (by volume) of 7 parts sand to 3 parts compost which was spiked with a range of total Pb concentrations 100 to 10,000 mg/kg (according to the designed model in Fig. 1) dry weight of Pb in the form of PbO. Sand was used to allow for proper aeration. Potting growth medium was chosen to best meet the needs of plant roots of these species for air, water, nutrients and plant support. The nutrient rich compost combined with sand made an excellent growth medium for these plant species.

Spiking the growth media with Pb contaminant was done using carrier sand of 6 kg (DW) mass, which was dried and thoroughly mixed in a dry bucket with pre-dried PbO dried to constant weight (checked at 0, 12, 18 and 24 hour duration). Dry carrier sand was used to ensure proper mixing of the PbO and the sand. Lead oxide in the dry carrier sands were mixed with calculated mass of sand and compost for each concentration in a cement mixer until a homogenized mixture was obtained. This was used to make batches of growth media in range of 100 to 10,000 mg/kg (FW) Pb for the two plant species.

Five lots each of about 10 g of the mixed and spiked growth media were sampled to check the Pb concentration of the growth media. These portions were taken from randomly selected pots, dried in the oven at 110°C and milled using the tema mill. A mass of 0.25 g of the milled sample was used to determine Pb concentration and (homogeneity) of the contaminant at each Pb concentration level using the Atomic Absorption Spectrometer (AAS) after acid digestion by nitric and perchloric acids. Certified reference materials (CRMS), duplicates and reagent blanks were used for quality control.

Cells	1	2	3	4	5
A	1000	1000	1000	1000	1000
B	1000	1000	1000	1000	1000
C	1000	1000	1000	1000	1000
D	1000	1000	1000	1000	1000
E	1000	1000	1000	1000	1000

Fig. 1a. Homogeneous (HO) --- robust
mean=1000; HF=1.00

Cells	1	2	3	4	5
A	900	700	900	1100	900
B	1100	1100	1400	1400	1400
C	1100	700	1000	900	900
D	1100	900	1100	1800	900
E	900	1100	900	1100	700

Fig. 1b. Low heterogeneity (LH) -- Robust
mean =1029; HF=1.2

Cells	1	2	3	4	5
A	500	300	500	1100	500
B	1100	1100	2200	2200	2200
C	1100	300	1000	500	500
D	1100	500	1100	4000	500
E	500	1100	500	1100	300

Fig. 1c. Medium heterogeneity (MH) -- robust
mean=962; HF=1.99

Cells	1	2	3	4	5
A	300	100	300	1000	300
B	1000	1000	3000	3000	3000
C	1000	100	1000	300	300
D	1000	300	1000	10000	300
E	300	1000	300	1000	100

Fig. 1d. High heterogeneity (HH) ---robust
mean=947; HF=3.19

Fig. 1. Four models of *in situ* heterogeneity for pot trial simulating *in situ* heterogeneity. Each cell of each treatment represent a Pb concentration in mg/kg

2.3 Creating the Simulated Heterogeneity Design in Pot Trial

The Method used in this pot experiment was built on the understanding of [14] with significant modifications to the dimensions of pot trial equipment and identity of contaminant (Pb instead of Zn). However, the use of heterogeneity factor (HF) in the created design (Fig. 1) used in pot trial is a novel aspect of this study (details of HF already published in [46].

A customized cell divider made from a 1 mm clear polyethylene terepthalate glycol (PTEG) sheet was inserted into the pots to produce a 5 by 5, 2-dimensional grid with each cell measuring 25 mm square and 170 mm deep. This was used to create the designed heterogeneity models. Labelled paper liners were inserted into each cell while filling cells with growth media. It provided a filling template, helped to maintain the structural integrity of the divider and minimized spillage from adjacent cells.

The gap between the paper liners and the outer edge of the pot were packed with an inert Sinclair Perlite (grain size 2.0-5.0 mm) because of the non-vertical sides of pots. Cells were filled according to the particular designed model of heterogeneity. Filling of the pots was done in two stages to ensure that equal volume of growth medium went into each cell and that the growth medium is evenly distributed throughout the pot. Further details on experimental method has been reported in an unpublished thesis [50].

The 80 completed pots (Fig. 2a) were placed in drip trays. Ten replicates of four treatments (homogeneous, low, medium and high heterogeneity) for each species were arranged on benches in the randomized block design (Figs. 2a and 2b).

Growth medium was moistened from below by capillary action before transplanting seedlings already established in a lead-free growth media for two weeks. Tap water was applied using a fine rose watering can minimising any disturbance to the pattern of heterogeneity.

The percentage moisture content of the growth medium was originally 8.5%. The pH of the growth media determined using pH meter {model: Hanna 209}, was 6.44±0.05. The established seedlings of the selected plant species *Brassica napus* (PI 601261) and *Brassica juncea* (PI 182921) were transplanted into the centre of each treatment after two weeks of growing in the lead-free growth media. Pots were maintained in the greenhouse for six weeks under simulated sunlight using light-emitting diodes (LED) lights (under a photoperiod of 12 hours) at 20±5°C. Plants were 60 days post-germination at harvest. Roots were carefully washed to remove soil particles that could introduce potential bias in measurements of metal concentration. Harvested roots and shoots were dried at 60ºC for 48 hours in a fan oven, weighed for DW, and analysed for Pb concentration using an Atomic Absorption Spectrometer (AAS) after acid digestion using nitric and perchloric acids [51] with matching analytical quality control.

Data were analysed using statistical software Minitab 16 and SPSS 21 for Windows. Statistical tools such as analysis of variance (ANOVA), Tukey post-hoc test and the mixed model ANOVA (treatment used as fixed factor and block as a random factor) were used to test for significance of measured variables for each species whilst, Kolmogorov-Smirnov test was used to test for a normal distribution of data. The Tukey post-hoc test was employed for the comparisons between concentrations in which a shared letter of the alphabet indicates that the mean values are not significantly different.

2.4 Root Placement Experiment

Root extraction device and pot divider were constructed as described in earlier work on zinc heterogeneity by [14] with modifications to dimensions of the pot equipment and methods. The root placement equipment such as the customized metal blade, holding block and root extraction device are described in an unpublished thesis [50].

Roots were extracted by placing each of the 40 cubes of growing medium in a wooden box and the customized sleeve removed. The cube was held securely in position with a holding block. A customized metal blade was used to divide growth medium into the 25 original individual cells, using measured grooves on top of the holding block. Roots were harvested from each cell using a root extraction device and the dry biomass recorded.

Harvested roots were weighed and dried in a fan oven at 60ºC for 48 hours. Dried, extracted roots from each cell were weighed and roots from cells of same nominal Pb concentration were combined, milled (using an herbage mill) and analysed for Pb. The recorded mass of root biomass was subsequently combined (mathematically) for the measurement of total root biomass in each pot. The raw measurements were used to assess root distribution in response to heterogeneity.

3. RESULTS

Both plant species survived until harvest. Visible signs of chlorosis, such as loss of green colouration, were observed in the heterogeneity treatments both species (Figs. 3a and 3b). Curly thinner stems, random turning of stem clockwise or anticlockwise to corners of pots and reduced leaf area were observed in *B. juncea* in low, medium and high heterogeneity treatments with severity in the HH treatment, which was absent in the homogeneous treatment (Fig. 3b). *Brassica napus* had broader leaves and sturdier stem than *B. juncea* in all treatments. Condition of plants at harvest is shown in Figs. 3a and 3b.

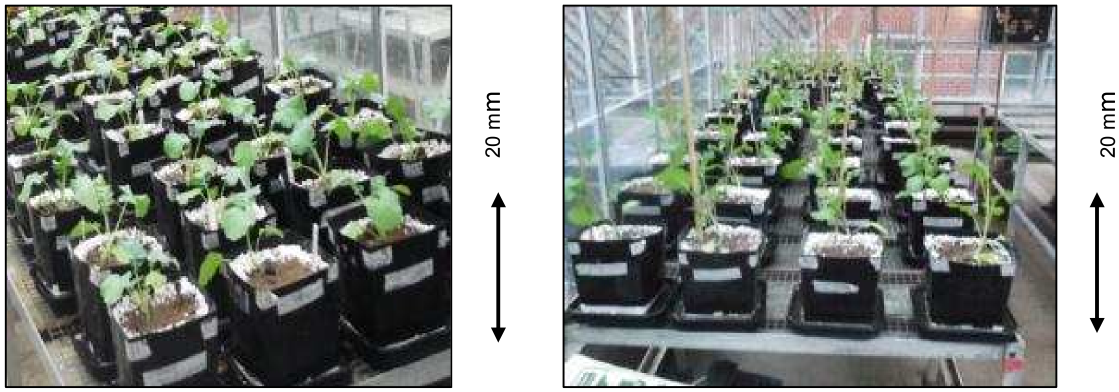
3.1 Biomass Results

Biomass generally decreased with increasing heterogeneity (Fig. 4a) with a 70% decrease in total dry biomass of *B. napus* in HH when compared to the HO treatment. However, the MH treatments had significantly higher total dry biomass (2 and 4 fold higher), when compared to LH and HH treatments respectively. A similar trend was observed in the shoot and root (Fig. 4a).

Similarly for *B. juncea*, biomass decreased with increasing heterogeneity with 90% lower total dry biomass in the HH treatment than the HO treatment (Fig. 4b). Shoot and root biomass in the HH were 50% lower than in the HO treatment. The LH treatment had significantly higher total dry biomass (1.7 and 2.3 fold higher) than the MH and HH treatments respectively.

The highest total biomass was produced in the homogeneous treatments for both species.

This difference in the total dry biomass between treatments was statistically significant ($F_{3,\ 36}$= 917.05; P< 0.05 and $F_{3,\ 36}$= 201.64: P< 0.05) for *B. napus* and *B. juncea* respectively. ANOVA and Tukey HSD test also showed a statistically significant difference between the 3 heterogeneity treatments. The mixed model ANOVA for each species showed a statistically significant effect (P < 0.05) of spatially heterogeneous Pb treatments on plant biomass. This is an indication that the degree of spatial heterogeneity of Pb had an impact on the biomass of both species.



(a) (b)

Fig. 2. Plants arranged in randomized block design showing (a) *B. napus* and (b) *B. juncea*. (Scale bar: 15 mm represents 20 mm) and (b) *B. juncea* (Scale bar: 9 mm represent 20 mm). Arrows represent scale bars

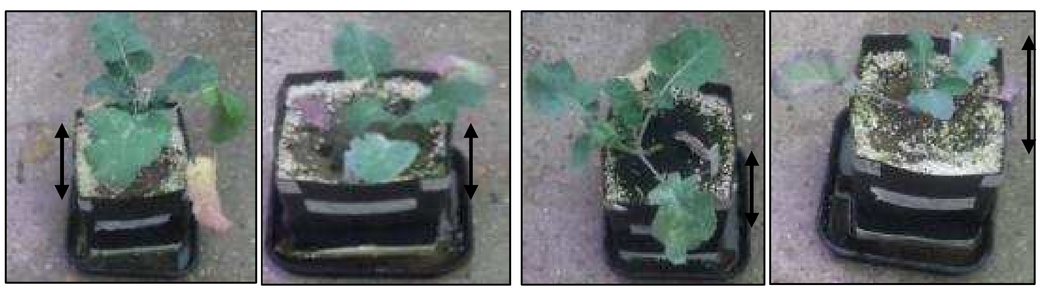
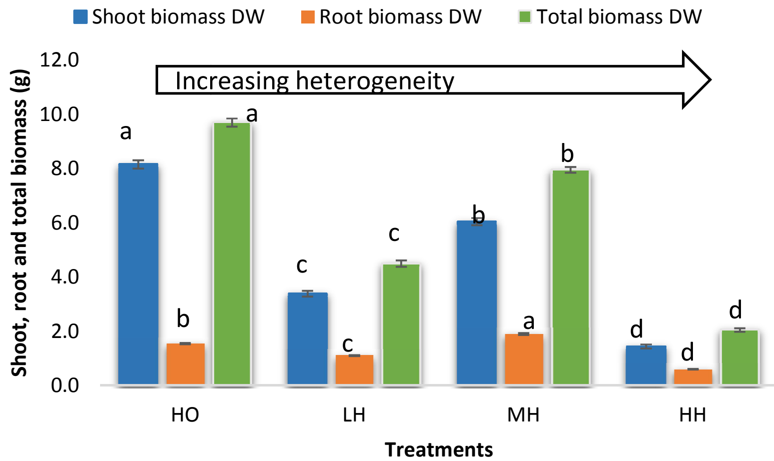
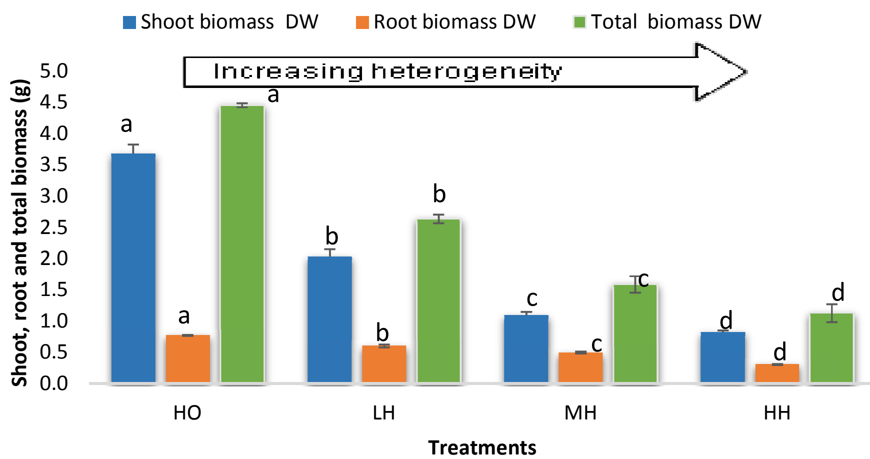


Fig. 3a. *B. napus* in order of increasing heterogeneity (HO, LH, MH & HH){scale bar- represents 10 mm for HO, LH, MH and HH respectively}from left to right generally showing decreased in biomass except with increasing heterogeneity, except for the MH treatment.

Fig. 3b. *B. Juncea* in HO, LH, MH & HH treatments from left to right (Scale bar: 42 mm represents 500 mm; Scale bar: 48 mm represents 800 mm; Scale bar: 49 mm represents 900 mm; Scale bar: 51 mm represents 1000 mm for HO, LH, MH and HH respectively) showing narrower leaves, slimmer stem in LH, MH & HH (decreased biomass) compared to HO. Key: Black arrows represent scale bars for each Figure with scale bar information below Fig.



(4a)



(4b)

Fig. 4. Mean shoot, root and total dry biomass in four treatments for (4a) *B. napus* and (4b) *B. juncea*. Means that do not share the same letter are significantly different as judged by the Tukey Post hoc test for each variable. Error bars represent 1 standard error on the mean, n=10

3.2 Lead Uptake Results

Shoot and root uptake for both plant species are presented and discussed in terms of both Pb concentration (mg/kg of dry plant biomass) and Pb mass per plant (in µg) as used in previous work [52]. Both methods of quantifying uptake showed varied effects on the plant from different perspectives. Lead concentration (in mg/kg) is often used in the estimation of human exposure to Pb and concentration of contaminant in herbage and soil, while the mass concentration (in µg), which compensates for simultaneous changes in both biomass and concentrations, finds useful application in estimating uptake for phytoremediation purposes. Both were used in this research because of the potential implications of this study for both human risk assessment and phytoremediation of Pb contaminated land.

3.2.1 Pb uptake results for *B. napus* and *B. juncea* expressed as Pb concentration (mg/kg)

The shoot and root Pb concentration in mg/kg increased with increasing heterogeneity with a peak uptake in the LH treatment, for *B. napus* whilst, *B. juncea* had peak shoot and root uptake in HH and MH treatments (Figs. 5a and 5b). However, the trend of increase in the shoot and root Pb uptake differed between the two species. The MH and HH treatments of *B. napus* were not significantly different in their Shoot uptake (mg/kg) concentration, while the LH and HO treatments were significantly different (Fig. 5a). The reverse as the case for *B. juncea*, the HO and the LH treatments were not significantly different in their root Pb uptake (mg/kg) concentration, whilst the HH and MH shoot Pb were significantly different. This is an indication to species specific differences between both species.

3.2.2 Uptake expressed as Pb mass (µg) for *B. napu s*and *B. juncea*

The shoot and root Pb mass (µg) were 40-80% lower in the HH treatment than the HO (Figs. 6a and 6b) for both species. The shoot Pb masses were maximum in HO where the highest shoot biomass was recorded.

The root Pb mass increased with increasing heterogeneity with a peak uptake recorded for MH treatment, which was 45% higher than HO root Pb mass (Figs. 6a and 6b). The root Pb mass had peak uptake in the MH treatment which was 40% higher than the HO treatment for both *B. napus* and *B. juncea*. The varied dry mass of shoot and roots recorded at harvest had influenced the uptake when expressed in terms of Pb mass.

The lowest mean shoot and root Pb mass were recorded in the HH treatments compared to the other spatially heterogeneous treatments (LH & MH) and the HO treatment.

Statistically significant differences ($F_{3,\ 36}$=164.38; 259.50 P< 0.05 and $F_{3,\ 36}$= 55.37; 116.40 P< 0.05) in shoot and root Pb masses were detected between treatments for *B. napus* and *B. juncea* respectively. The Mixed model ANOVA results independently for each species also showed a significant (P< 0.05) effect of spatial heterogeneity on Pb uptake. This clearly suggest that the degree of spatial heterogeneity had an impact on the extent of Pb uptake of both species.

3.2.3 Shoot and Root Concentration Factors (CF_{shoot} and CF_{root}) of *B. napus* and *B. juncea*

The shoot and root concentration factors (CF_{shoot} and CF_{root}) {Pb concentration in shoot or root (mg/kg DW)/mean soil concentration of Pb of *B. napus* and *B. juncea* are compared in Fig. 7 below.

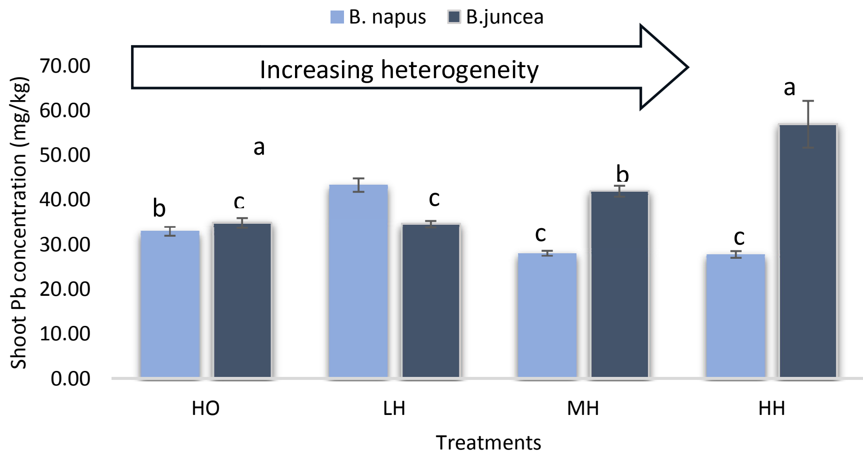
Generally, CF_{shoot} for *B. napus* and *B. juncea* ranged from 0.03 to 0.06 with 1.7 and 1.5 fold rise in LH and HH treatments, when compared to the HO treatment respectively (Fig. 7a). This implies that higher amount of Pb was accumulated in the roots of these species in the LH and MH treatments when compared to the other treatments. Shoot concentration factors in all treatments were below the accumulator threshold of 1, by 33-70% for *B. juncea* and *B. napus* respectively.

Brassica juncea had 1.5 fold higher CF_{shoot} in the HH treatment when compared to the HO treatment whilst *B. napus* had similar CF_{shoot} in the HH as the HO treatment. This is an indication of increased shoot uptake (mg/kg concentration) of *B. juncea* in the HH treatment, whilst *B. napus* might generally exclude Pb from the shoot in all treatments with an exception of the LH treatment for both shoot and roots.

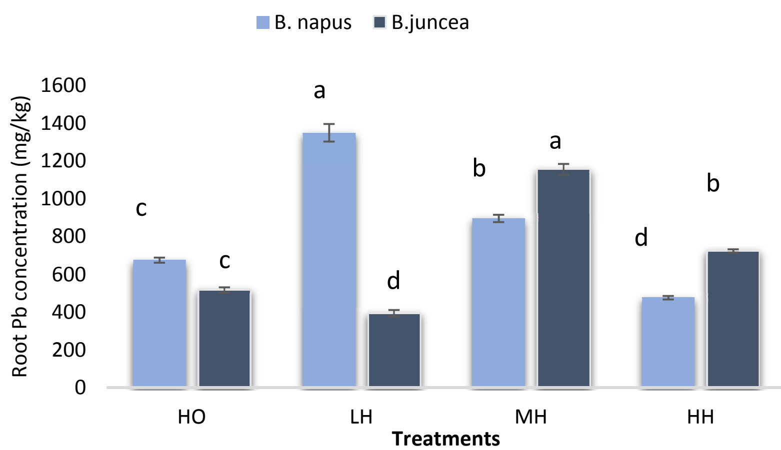
The root concentration factors (CF_{root}) of both species ranged from 0.41 to 1.41 (Fig. 7b). The CF_{root} of *B. juncea* increased with increasing heterogeneity with 60% lower CF_{root} in the HH treatment, when compared to the peak CF_{root} in MH treatment. The CF_{root} of *B. juncea* in the HH was 40% higher than that of the HO treatment and 60% lower than the peak CF_{root} in MH treatment.

Brassica napus had a peak CF_{root} in the LH treatment which was 2 fold higher than the HO CF_{root}. However, CF_{root} was higher by factors of 1.4 and 2.8 in the HH treatment when compared to the HO and LH treatments of *B. juncea* respectively. This decreased CF_{root} of both species in the high heterogeneity treatment, when compared to the peak CF_{root} supports earlier findings for Zn (Thomas, 2010) of reduced root CF (10 fold decrease) in highly heterogeneous Zn treatment with *B. napus* and *juncea.*

A significant variation (P = 0.000 < 0.05) in CF was observed between treatments for both species. This suggests that the extent of spatial heterogeneity had a significant effect on the ability of plants to accumulate or exclude Pb.



(5a)



(5b)

Fig. 5. (5a) Shoot and (5b) Root Pb concentration (mg/kg) between treatments of *B. napus* and *B. juncea*. Means that share the same letter are not significantly different as judged by the Tukey Post hoc test for each treatment and species. Error bar represent 1 standard error on the mean, n=10

3.2.4 Root placement results for the more realistic *in situ* heterogeneity experiment

Typical root morphology for the two species are shown in Figs. 8a and 8b.

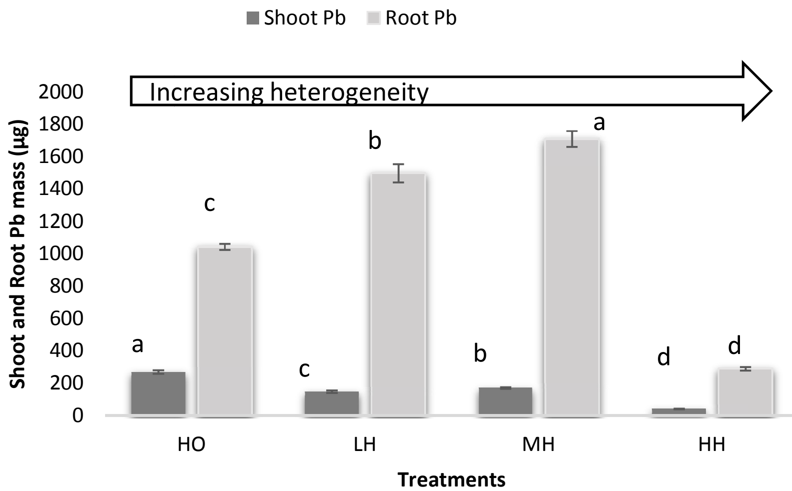
3.3 Root Response Result for *B. napus*

Figs. 11a and 11b below show the mean root biomass DW in cells within the same concentric patches {outer, middle and central} as shown in (Fig. 10) with same nominal soil Pb concentration of *B. napus*.

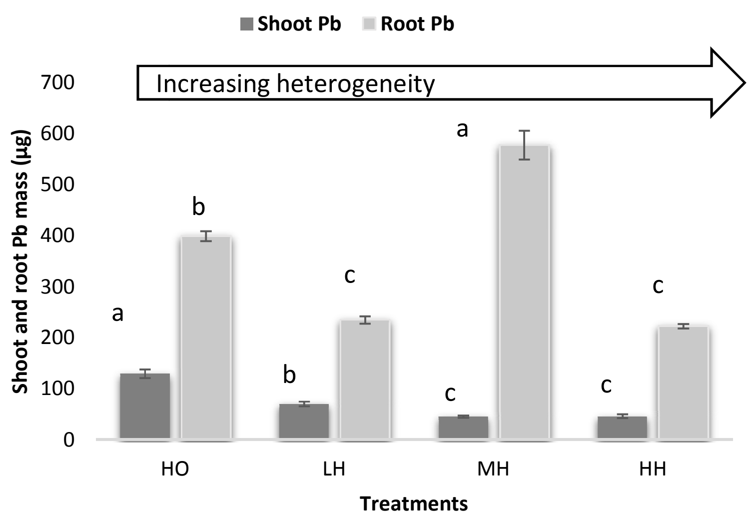
The ANOVA result show significant differences ($F_{9, 90}$ = 585.02; P = 0.000 < 0.05) between root biomass DW in concentric patches of varied soil Pb concentration. The 1000 mg/kg concentration in the central patch had 13 to 94% higher root biomass, when compared to the all patches with the same nominal Pb concentrations.

Root biomass of the 300 mg/kg outer and middle patches were significantly different (T= -8.60; P =

0.000 < 0.05) with the outer having 10% higher biomass than the middle patch. The root biomass of the 100 and 300 mg/kg outer and middle patches did not differ significantly (T = 1.48; P=0.154 > 0.05). Patches with higher Pb concentrations (3000 and 10000 mg/kg) were not significantly different in their root biomass (T= 2.24; P=0.066 > 0.05). However, they differed significantly from the 100, 300 and 1000 mg/kg patches in their root biomass. The root biomass has been decreased by 17 to 90% in the patches with higher Pb concentrations (3000 and 10000 mg/kg). This is an indication that the root biomass had been impacted on by the patch contrast (heterogeneous distribution of Pb in cells).

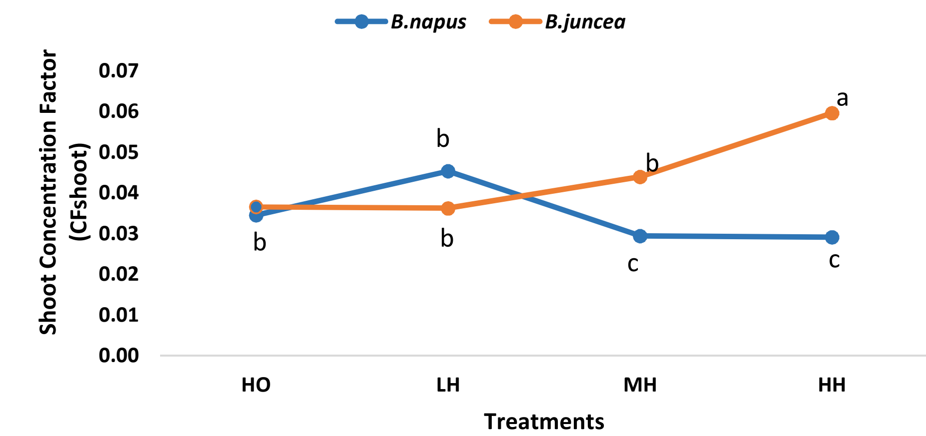


(a)

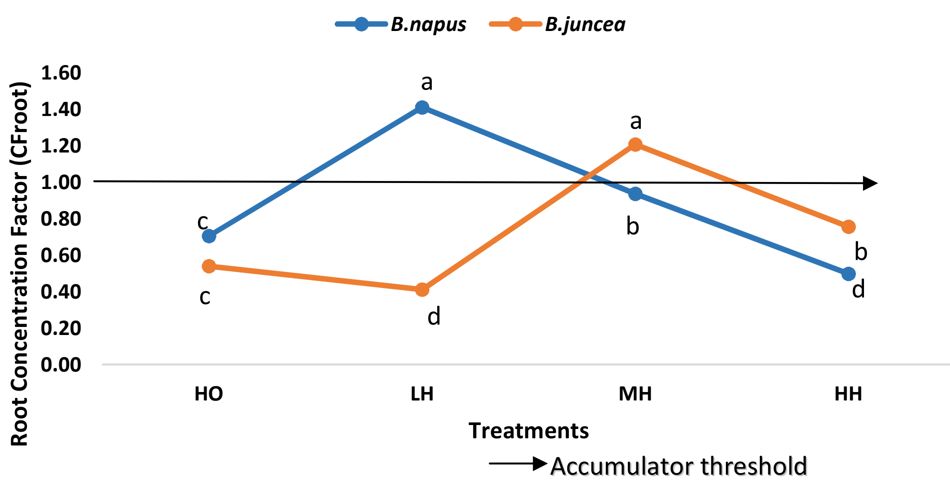


(b)

Fig. 6. Shoot and root Pb masses (µg) between treatments of (6a) *B. napus* and (6b) *B. juncea*. Means that share the same letter are not significantly different as judged by the Tukey Post hoc test for each variable and treatment. Error bar represent 1 standard error on the mean, n=10



(a)



(b)

Fig. 7. (7a) Shoot and (7b) root Concentration Factors for *B. napus* and *B. juncea* grown in 4 different treatments of Pb spatial heterogeneity. Where CF is greater than 1 (accumulator threshold) Pb is accumulated. Means that share the same letter are not significantly different for each species as judged by the Tukey Post hoc test

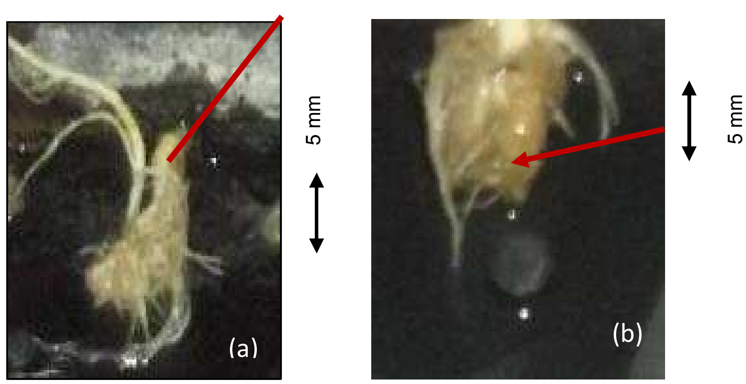


Fig. 8. Extracted roots showing (a) a tap root in C3 of *B. napus* and (b) a fibrous root in *B. juncea*. (Scale bar--4 mm represents 5 mm)

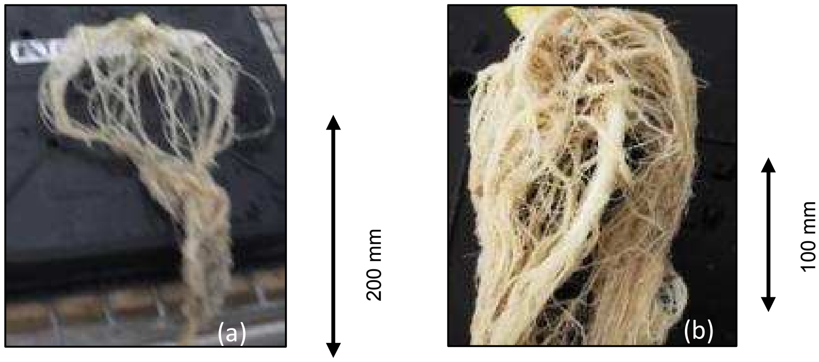


Fig. 9. Roots of (a) *B. juncea* (scale bar- 2.9 mm represents 200 mm) with no tap root and that of (b) *B. napus* (scale bar--- 2 mm represents 100 mm) showing a central tap root from an earlier pot trial using the simplistic binary heterogeneity reported in an unpublished thesis [50]

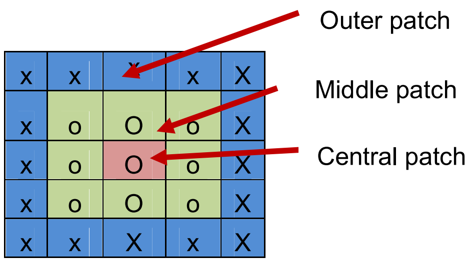


Fig. 10. An illustration of concentric patches

There was a trend (R^2=0.996) of decreasing root biomass with increasing patch soil Pb concentration in the outer concentric patches (Fig. 12a). However, there was no significant linear relationship (R^2=0.2364) between decreasing root biomass (Fig. 12b) with increasing patch Pb concentration of the middle concentric patches.

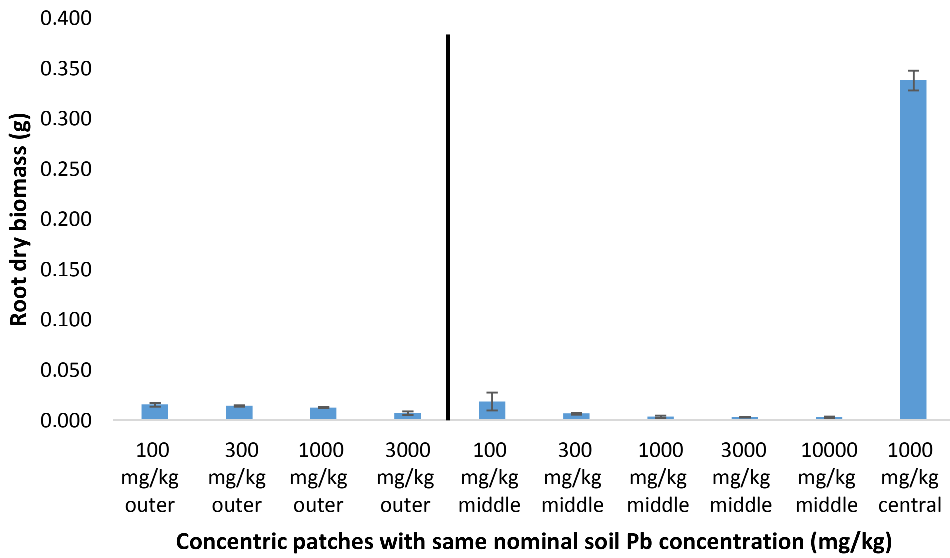
The root Pb concentration in patches with same nominal concentration increased with increasing nominal soil Pb concentration of patches (Fig. 12a). There was a strong positive relationship (R^2 = 0.98) between cell root Pb concentration and the nominal soil Pb concentration with the ~99% of the variance accounted for by the regression model of cell root Pb concentration against nominal soil Pb concentration (Fig. 12b) which suggest that the soil Pb concentration had an impact on root uptake in patches. It is also an indication this plant species responded to the increasing root uptake of Pb by decrease in the proportion of root biomass in patches with high Pb concentration.

Fig. 13 below show the root biomass DW in patches with same nominal soil Pb concentration of *B. juncea*. The ANOVA result also showed significant differences (F = 13.77; P = 0.000 < 0.05) between cells of varied soil Pb concentration. The 1000 mg/kg central patch had 6 to 98% higher root biomass, when compared to the other concentric patches with same nominal soil Pb concentrations.

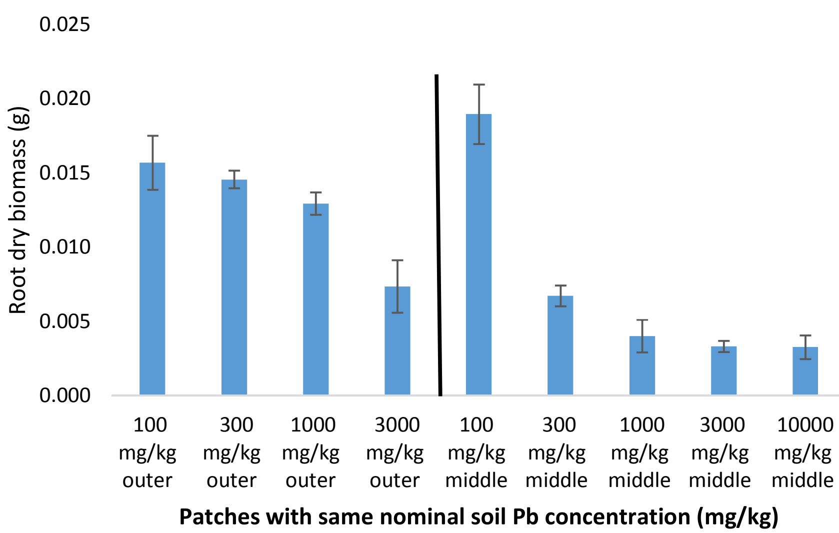
The 100 and 300 mg/kg did not differ significantly (T= -0.80,-0.28; P=0.445, 0.784 > 0.05) in their outer and middle root biomasses with 50 and 5% higher root mass in middle and outer patches of the 100 and 300 mg/kg respectively.

Patches with higher Pb concentrations (3000 and 10000 mg/kg) were significantly different from the 100, 300 and 1000 mg/kg outer and middle patches in their root biomass. The 100, 300 and 1000 mg/kg patches were similar in the distribution of root in the outer and middle patches. The root biomass was decreased by 11 to 95% in the patches with higher Pb concentrations (3000 and 10000 mg/kg). Result showed that the root mass of *B. juncea* also decreased with increasing soil Pb concentration of cells which implied an ability of the root to detect patch contrast and contaminant heterogeneity. There was a trend (R^2=0.7050 and 0.6589) of decreasing root biomass with increasing patch Pb concentration in the outer and middle concentric patches respectively (Fig. 14b).

A similar trend of increased root Pb concentration with increasing patch nominal Pb concentration was also observed in *B. juncea* (Fig. 14a). Cell root Pb concentrations did not differ significantly between cells with 100, 300 and 1000 mg/kg Pb concentrations. There was a strong positive relationship (r^2=0.97) between cell root Pb concentration and the nominal soil Pb concentration with 97% of the variance accounted for by the regression model of cell root Pb concentration against nominal soil Pb concentration (Fig. 14b). This implies that individual cell root uptake has been influenced by the soil Pb concentration in the HH treatment. The slope of the regression model shows that root Pb uptake in *B. juncea* was approximately 2 times higher than that of *B. napus*.



(a)



(b)

Fig. 11. (a) Root biomass DW in concentric patches of same nominal soil Pb concentration of *B. napus* in the HH treatment with the central 1000 mg/kg patch (b) Root biomass DW in the outer and middle concentric patches with same nominal soil Pb concentration of *B. napus* in the HH treatment without the central 1000 mg/kg patch

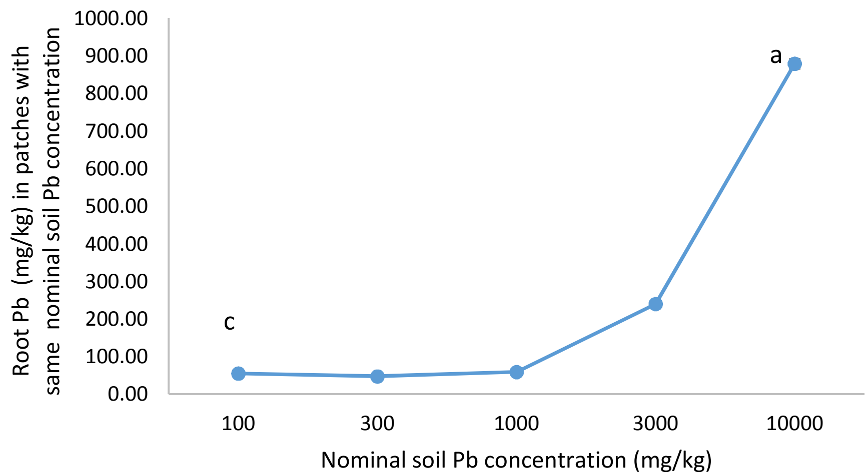


Fig. 12a. Root Pb concentration between nominal soil Pb concentrations of *B. napus* in the HH. Means that share a letter are not significantly different as judged by the Turkey Post hoc test. n=10

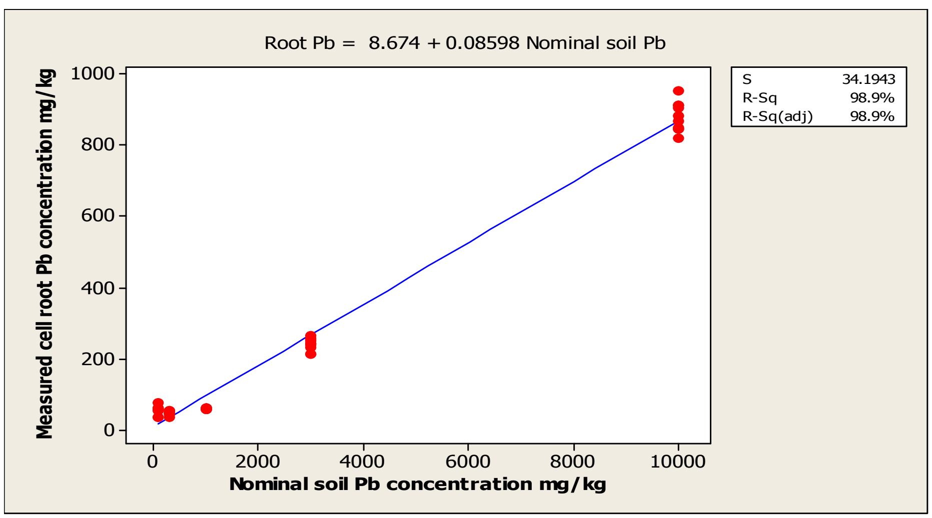


Fig. 12b. Regression model of measured root Pb concentration of patches with same nominal soil Pb against nominal soil Pb concentration of *B. napus* in the HH treatment.
Root response result for *B. juncea*

3.3.1 Comparison of plant response to the field modelled heterogeneity

These results suggest that plant roots for both species have shown a response to changing heterogeneity. It also showed similarities in response to changing heterogeneity between the two plant species, though significantly impacted to varied extent. However, both plant species were significantly different (T = 2.68; P = 0.009 < 0.05) in the proportion of root mass allocated to cells of varied Pb concentration. Visible differences were observed in the root morphology of these species. Fig. 15 compares the root mass of the 1000 mg/kg central patches of both species. The total root mass of *B. napus* and *B. juncea* varied by factor of 2. Result suggest that *Brassica napus* had 53% higher root biomass in the central patch than *B. juncea.* This difference was statistically significant (T = -28.26; P = 0.000 < 0.05). This contrast in root morphology is due to the presence of tap root in *B. napus* and its absence in *B. juncea*. The differential root structure and morphology of these species might have contributed to the varied extent of the effect of spatial heterogeneity.

4. DISCUSSION

Our results demonstrate the critical role that realistic heterogeneity plays in the growth and contaminant uptake by plants growing in contaminated environments. The understanding of the spatial distribution of Pb in soil is important for making more accurate predictions of Pb uptake by plants, and therefore for the assessment of potential human exposure by ingestion. It is also useful in the development of a more effective phyto-remediation strategies for Pb contaminated land.

Results showed that differing levels of heterogeneity have a significant effect (between 60 and over 100%) on both uptake and biomass between treatment and species. For *B. napus*, there were significant differences in both biomass and Pb uptake between treatments. Shoot and root dry biomass decreased with increasing heterogeneity with a peak biomass in the medium heterogeneity treatment. The shoot and root biomass in the homogeneous treatment was significantly higher (5 fold), than the high heterogeneity treatment. Similarly, significant differences in biomass were also found between treatments for *B. juncea*. Shoot and root biomass decreased with increasing heterogeneity with maximum biomass in the homogeneous treatment which was 4 fold higher than the high heterogeneity treatment.

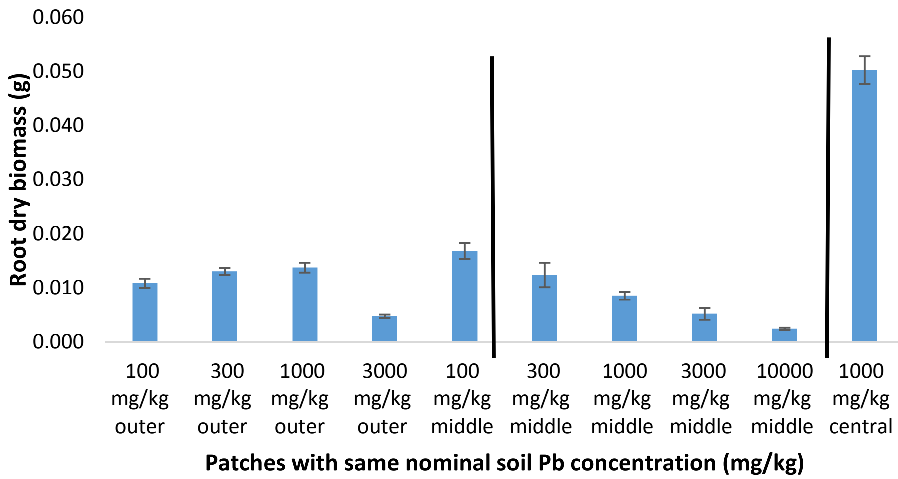


Fig. 13. Root biomass DW in concentric patches of same nominal soil Pb concentration of *B. juncea* in the HH treatment

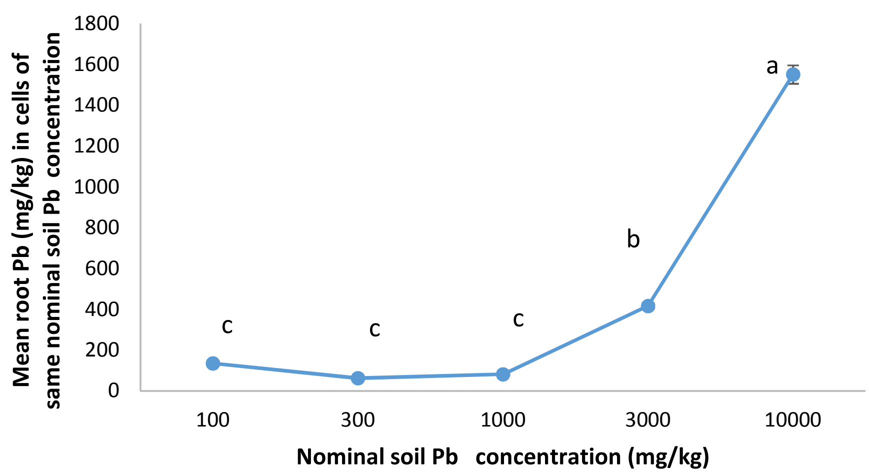


Fig. 14a. Mean root Pb concentration between nominal soil Pb concentrations of *B. juncea* in the HH treatment. Means that share a letter are not significantly different as judged by the Turkey Post hoc test. n=10

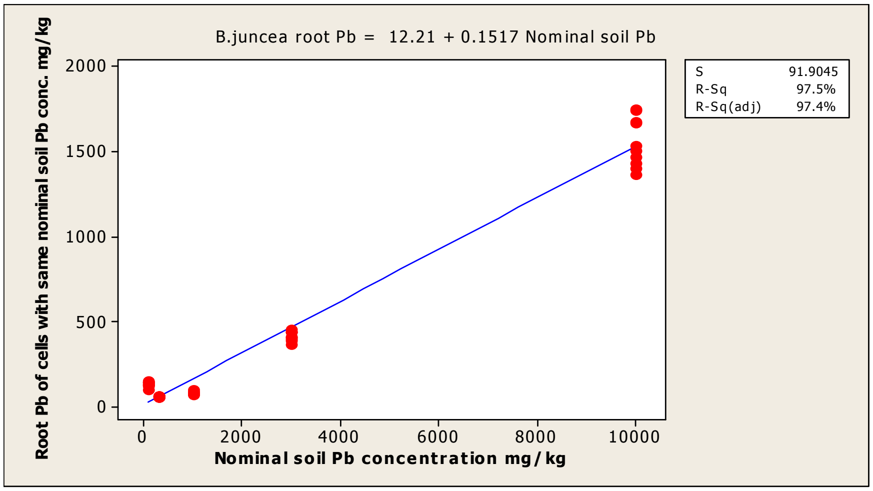


Fig. 14b. Regression model of mean root Pb concentration (Patches of same nominal soil Pb) against nominal soil Pb concentration of *B. napus* in the HH treatment

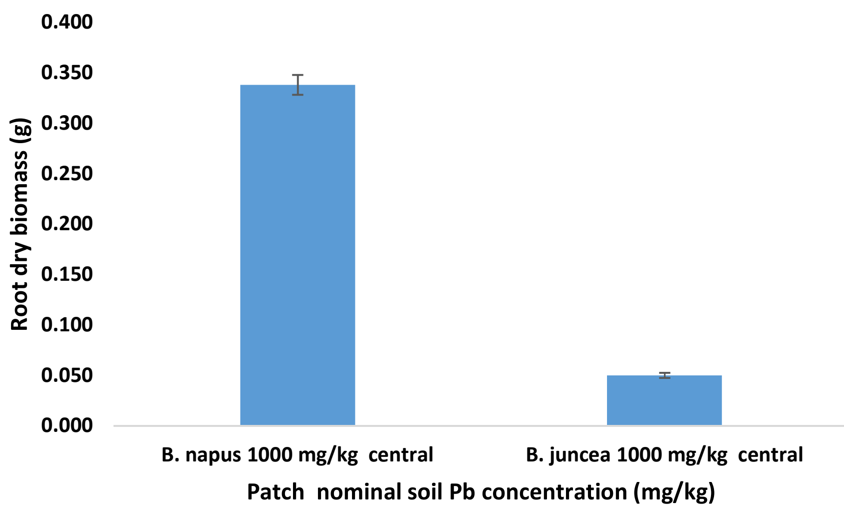


Fig. 15. Comparison of the central root biomass of *B. napus* and *B. juncea*

B. napus and *B. juncea* generally showed a decreased biomass with increasing heterogeneity. The root mass in patches with the same Pb concentration also varied for both species in the high heterogeneity treatment. This is similar to findings of [28] of decreased root mass in heterogeneously Zn contaminated loamy soil.

The biomass and uptake results have implications for phytoremediation. Earlier Studies [53,12-14] reported that the effect of heterogeneity of Cd and Zn in soil on plant biomass and uptake. Findings from this study suggest that predictions of plant uptake and plant performance for use in phytoremediation and risk assessment from homogeneous pot trials may not be accurate representations of plant uptake and performance under field conditions, which are usually heterogeneous to some extent.

Uptake of Pb in these species varied with increasing heterogeneity. Plant Pb uptake (Concentration in mg/kg) peaked in the low heterogeneity treatment for *B. napus* and in the high and medium heterogeneity treatments for shoot and root of *Brassica juncea* respectively. Uptake expressed in Pb mass (µg) showed a different trend from that expressed as Pb concentration for both plant species. This is because the Pb mass accounts for the simultaneous changes in biomass and concentration. The shoot Pb mass peaked in the homogeneous treatment, where the highest biomass was recorded for both species. A different trend was observed in the root Pb mass, which peaked in the medium heterogeneity treatment for both species. This is an indication that the increased plant yield (biomass) in the above-ground part (i.e. shoot biomass) in the homogeneous treatment has influenced the shoot Pb mass. It also suggests that *in situ* heterogeneity of Pb may produce higher root Pb mass (e.g the medium heterogeneity treatment), which could affect the overall total plant Pb mass, depending on the level of heterogeneity.

However, uptake increased with increasing heterogeneity for *B. napus* with the exception of the HH treatment where lower Pb concentration (mg/kg) and mass (µg) were observed. This confirmed similar findings of increased uptake of Zn with increasing heterogeneity for *B. napus* [14]. *Brassica juncea* had lower Pb uptake (expressed as Pb mass) with increasing heterogeneity with an exception of the MH treatment where a significantly higher uptake was recorded. This is in contrast to earlier findings for Zn [14] of increased uptake of Zn with increasing heterogeneity for *B. juncea*. The differences in peak uptake and biomass between treatment and species is a pointer to the species specific differences in uptake rate at varied level of Pb heterogeneity.

The high heterogeneity treatment had significantly lower shoot and root Pb mass, when compared to the peak Pb mass found in the medium heterogeneity and homogeneous treatments. This exceptional difference in Pb mass of MH treatment might have been influenced by the biomass, scale of heterogeneity, size of the root ball size and root response to the spatial distribution of Pb. The root response result showed that high proportion of roots is proliferated in low Pb concentration patch. This suggests that plants with low biomass growing on contaminated land with highly heterogeneous distribution of Pb is expected to have low Pb mass. The low biomass in the high heterogeneity treatment has resulted in reduced Pb uptake expressed in terms of Pb mass.

The shoot concentration factor (CF_{shoot}) of both species were generally well below the accumulator threshold, and was~ 20 to 25% lower than the root Pb uptake. However, HO, LH and HH treatments had CF_{root} below the accumulator threshold. Results showed that both species could exclude Pb at nearly all levels of heterogeneity to varying extents, with peak Pb uptake in the medium and low heterogeneity treatments for *B*. *napus* and *B*. *juncea* respectively. This indicates that they possess strong mechanism for excluding heavy metals from their tissues, as previously reported [14].

There is also an indication that both plant species will exclude more Pb from their roots in the highly heterogeneous treatment (with a decrease in CF_{root} in the HH treatment by factors of ~ 3 and 2, when compared against the peak CF_{root} in LH and MH treatments of *B*. *napus* and *B*. *juncea* respectively). A similar trend of decreased CF_{root} has been reported in earlier studies [14] with *Plantago lanceolata* in Zn spatial heterogeneity treatments. However, *B*. *juncea* had higher CF_{shoot} and CF_{root} (by a factor of 1.5 and 2 respectively) in the HH treatment than *B*. *napus*. This suggests that the effect of spatial heterogeneity on uptake of Pb is species-specific. Higher concentration factors suggest that plants possess weak mechanisms to exclude heavy metals and so contaminant build up occurs in plant tissues especially in the roots, whilst lower CF implies a strong mechanism of exclusion. Studies by [54,55,14] suggest that the concentration factor (CF) increases where mechanisms that exclude contaminants are weaker and subsequently result in contaminant accumulation into plant tissues.

Exclusion [56,57] is also one of the five theories postulated to explain why plants take up Pb from the soil, despite its widely proven toxicity to plants. The others include tolerance, accumulation, indication and defence mechanism against herbivory. Plants species/varieties in this study exhibited exclusion, accumulation and tolerance to high Pb in the soil in some cases. Both species exhibited a combination of these traits with generally observed exclusion of Pb from the shoots.

The shoot concentration factor (CF_{shoot}) showed that about 4-5 times lower Pb was accumulated in the shoot than in the root for most plant species/varieties used. This is in line with previous works of [54,58]. [59] also reported that 90% of Pb is accumulated in the root and that increasing accumulation of Pb in the roots can cause some ultra-cellular changes within plant tissues. Symptoms of such ultra-cellular changes include chlorosis, wilting and death of the plant [59]. Severe chlorosis and wilting of leaves were observed in *B. napus*. Similar observations were made by [14] for Zn spatial heterogeneity on plant growth and uptake. However, the cause of these symptoms was not established in this study.

Results of this experiment also suggest that *B. napus* would be more sensitive to spatial heterogeneity than *B. juncea* and that *Brassica juncea* will therefore grow better than *B. napus* in soil that is heavily contaminated with Pb (i.e. > 1000 mg/kg) in a heterogeneous way. The reason for this sensitivity to spatial heterogeneity in *B. napus* is not known.

However, it could be partially attributed to its root morphology and size as earlier stated. Other factors might have influenced the different response of this species to treatments compared to *B. juncea* in this study and in earlier work with Zn. For example, variation in genetic, physiological or biochemical adaptations of plants to different contaminants might have influenced this plant response to Pb heterogeneity. [60-63] suggest that genetic, physiological and biochemical adaptations of different plant species could influence uptake, tolerance and response to contaminants in the soil. Other factors that could produce elemental variability or variation in plant response to contaminants in soil such as transportation and deposition of contaminants within plant tissues, developmental stages, seasonal variation and differences in microclimatic/micro edaphic conditions has been reported by [64-67].

Findings of this study provided an insight to the important role of spatial distribution of contaminants in metal uptake from the soil by plants, tolerance to contaminants in soil and growth and development in plants.

4.1 Root Response

An investigation of the root distribution and behaviour in the high heterogeneity treatment, also found some differences in response to more realistic heterogeneity between both species.

In this study, decreased root mass was recorded in patches with high Pb concentration, when compared with patches with low Pb. This suggest preferential proliferation of roots in low Pb patch. An earlier pot experiment with *T. caerulescens* in [24], suggested that some plant species could discriminate between patches with different Zn-contaminant concentrations within the range of their root system. A comparison of the root biomass of the central 1000 mg/kg patch of both species shows that *B. napus* had 76% higher root mass in the central cell than *B. juncea*. This supports the fact that the higher central root biomass in *B. juncea*, when compared to the other patches did not indicate a tap root as in *B. napus*. A network of fibrous roots were observed in *B. juncea* (Fig. 9a). This clearly supports the inference that the differential root morphology between species might have influenced root placement in patches of varied Pb concentrations.

The regression model of root mass in patches against nominal soil Pb concentration for both species showed that there was a near linear positive relationship between root Pb concentration and nominal soil Pb concentration, which implies that root Pb concentration increased as a function of soil Pb. The slope coefficient of this regression indicates that the the root-Pb concentration is present at ~9% and 15% total Pb concentration of the growth medium. The experimental determined percentage extractable Pb was 18 % of the total Pb, added as PbO (reported in an unpublished thesis—[59]). This suggest that *B. napus* might have excluded approximately half of the bioavailable Pb while *B. juncea* accumulated around 80% of the bioavailable Pb. However, this is a rough estimate of the plant Pb uptake. It is an indication that the bioavailable pool of contaminants in the soil may not be completely taken up by plants and the amount taken up is partially dependent on plant species. The percentage extractable Pb in this study is higher by a factor of ~2, when compared to the predicted 10% of bioavailable Pb in the soil to plants reported by [68], but similar to reported values of 19 and 21% by other workers [69,70] respectively. This result suggest that percentage bioavailable Pb to plants could be higher than earlier predicted values, although there are other factors to consider. Bioavailability of Pb in soil to plants may be influenced by a number of factors. From literature (e.g, [1], it is known that the bioavailability of Pb in soil to plant may be influenced by soil type, pH and Pb speciation (e.g. PbO in these pot trials) and of course, the estimation may also vary with the reagent used for extraction.

The differences in root behaviour to Pb spatial heterogeneity and placement of roots in a heterogeneous distribution of contaminant might also have contributed to their varied response, for example, the peak effects observed at different levels of heterogeneity. Further work in this area may provide useful insights that could help enhance the success of phytoremediation.

The root response result also indicated strongly that responses to heterogeneity might be due to the nature, morphology and size of the root ball. A central tap root (Fig. 9b) was observed in *B. napus* variety used in this study, but was absent in *B. juncea*. The *Brassica juncea* variety used had several branched fibrous root networks.

Similarly, earlier studies by [14] suggest that possible root proliferation in response to patchy distribution of Zn in a pot trial. Results indicated that the variation in the response of these plant species to the different treatments might be due to the different pattern of root allocation to resources and contaminants. However, it was opposed to the foraging habit observed for Zn in *Thlaspi carulescens* in previous studies by [24].

Root placement in the high heterogeneity treatment provided further insight into the behaviour of these plant species in heterogeneous media. Decreased root mass was observed in patches with increased soil Pb concentration which suggest that both plant species were able to detect patch contrast (heterogeneity) of Pb in the growth medium. Increased root Pb concentration with increasing soil Pb concentration of each cell, with the highest root Pb concentration in the 10000 mg/kg concentration is an indication that the contrast in Pb distribution across cell in high heterogeneity treatment could influence root Pb uptake in each cell and subsequently the overall Pb uptake with impact on the proportion of roots. This might have partially influenced the instances of peak uptake at different levels of heterogeneity in this study.

The similarities in how these species respond to heterogeneity despite their contrasting root morphology suggest an avoidance response to the toxicity of Pb irrespective of root morphology. However, it will take a multidisciplinary approach often involving physiological and biochemical investigations to confirm this inference and to provide further insights on varied plant response.

The root placement found three distinct regions of root distribution as a function of soil Pb concentration in both species. The highest proportion of *B. napus* roots (26 to 113%) were proliferated in the 1000 mg/kg central patch, when compared to the other patches. Results show that roots were selectively placed in the low Pb patches (100 and 300 mg/kg patch) with approximately 80% of roots proliferated in the central 1000 mg/kg patch. This is an indication that the tap root which was located in the central cell (C3) of *B. napus*, where greater proportion of the roots were placed had influenced the overall mass of roots in the 1000 mg/kg patches. This suggests that the tap root is a big proportion of root mass and might have influenced the proportion of root proliferated to other patches in response to spatial heterogeneity. It is also an indication that the contaminant concentration and the range at which these patches are located in relation to the root could play an important role in determining the distribution of roots into patches with similar or different contaminant concentrations.

There was a continuous decrease in root biomass (~16 to 90 %) with increasing soil Pb concentration in the middle patches of *B. juncea*. The heterogeneity design (Fig. 8) shows that cells with elevated Pb concentrations (patches with 3000 and 10000 mg/kg) were located in the middle patch. It is a pointer to the fact that these patches with elevated Pb concentrations might have influenced the proportion of roots proliferated in the middle patches with an exception of the 100 mg/kg middle patch of *B. napus*.

The 3000 outer patch had 20 to 80% lower root mass than the 100, 300 and 1000 mg/kg outer patches. Rather than a decreased root biomass in the outer 1000 mg/kg patch, the root biomass in the 100, 300 and 1000 mg/kg outer patches were not significantly different. It is an indication of the adaptation of this plant species to this Pb concentration range. The highest proportion of roots (10 to 50%) were proliferated in the 1000 mg/kg central, when compared to the other patches. This denser root at 1000 mg/kg represent the central cell (C3) where the seedling was transplanted originally and the additional root mass in *B. napus* is as result of the tap root. This supports the finding that root morphology of both plant species plays an important role in the root response of these plants to Pb spatial heterogeneity, particularly in the proportion of roots proliferated into patches. Results also show that roots were selectively placed in the low Pb patches (100 and 300 mg/kg patch).

However, the mechanism of root proliferation in cells is not yet understood. Studies by [71,72,21] suggested that the mechanism of root proliferation involve interactions between the roots and the soil, based on root functional architecture. Some previous studies expressed varied opinion on the mechanism of root response in heterogeneous media.

For example, [73] suggested that proliferation of roots into patches can be influenced by the density of the plant tissue. A Study by [74] suggest that root proliferation as a measure of increased root biomass does not give a complete picture of the change in the plant root system, as alterations in the architecture of root system can occur without a change in the biomass. [75] argued that the proliferation of roots in patches may be related to the specific root length (SRL) or root length per unit mass (cm/g), which varies with root diameter and often used as a substitute to root diameter will provide relevant insight into root response. [28] suggested that the use of neutron radiography to study live plant roots in heterogeneously contaminated soil may provide a source of valuable information to explain root response in pot experiments. Based on the nature of the root placement experiment, it was more practically possible to use the root biomass that has been widely used in previous studies [24,12,76,21,14]. Results show that these plants will preferentially proliferate roots in patches with low Pb concentration in response to Pb heterogeneity to avoid Pb toxicity.

It also indicated that *Brassica napus* and *Brassica juncea* are tolerant Pb accumulators using classification by [56] of three categories of plant in response to increasing metal concentrations namely, accumulators, indicators and excluders. [65,66] reported the ability of some plant species to resist Pb concentration build up in certain parts of the plants by reduced movement of contaminant across the plasma membrane through the formation of a callose barrier. However, it is not known if these species are capable of forming callose barriers in their roots.

Whilst both plant species have shown the ability to recognise Pb patch contrast, it is essential to note that *B. napus* tended to be more sensitive (by factors of 1.2 to 2) in detecting patch contrast than *B. juncea*. As mentioned earlier on in this discussion, varying plant response to spatial heterogeneity, and the behaviour of the roots in response to changing heterogeneity, might also be associated with the evolution of diverse complex mechanisms by these plants to withstand heavy metal stress in the soil.

This more realistic simulation of *in situ* heterogeneity reflects plant root response to more realistic patterns of heterogeneity of Pb that may be characteristic of field conditions.

4.2 Implications

A generic risk assessment criteria for contaminated land uses the relationship between the Pb concentrations in the soil and the plant, expressed as concentration factors, to estimate the potential human exposure and therefore the risk to human health from consumption of vegetables grown on contaminated land [77].

This study found that spatial heterogeneity of Pb had a significant impact on plant uptake and plant growth, compared against uptakes measured for homogeneous spatial distributions. This also has implications for plant uptake models used for estimation of human exposure to contaminants in risk assessment.

The results of this study suggest that uptake models based on more realistic field-modelled heterogeneity could therefore help improve the accuracy and reliability of risk assessment models used to estimate human exposure to toxic contaminants. It also suggest that concentration factors predicted by models that assume homogeneous distribution of contaminant in the soil are not realistic.

There is also an implication for phytoremediation of Pb contaminated sites. The uptake estimates based on homogeneous pot trials for phytoremediation will be equally inaccurate to those already discussed for human health risk assessment. Therefore, studies concerned with identification of suitable plant species for phytoremediation should take into consideration the spatial heterogeneity of contaminant in the field. This may be useful in making more effective selection of suitable plant species for specific contaminated sites. This study was conducted at a mean Pb concentration of 1000 mg/kg and only for *B. napus* and *B. juncea* which is a limitation to the study. Further studies with several other plant species at higher mean Pb concentration (e.g 5000 mg/kg) may be required to substantiate these findings. However, there is a potential implications for modifying the *in situ* heterogeneity to enhance uptake mass by physically changing *in situ* heterogeneity of Pb in soil at contaminated sites prior to phytoremediation through specifically designed ploughing to mix the soil to reduce the *in situ* heterogeneity. This will help increase the mass of Pb taken up by specifically chosen plant species (where they behave like these *Brassica* species at this Pb concentration), thus increasing the success of phytoremediation of Pb contaminated sites.

Based on the results of this research, it is recommended that this new technique of assessing the impact of different levels of heterogeneity be employed in research on plant uptake of contaminants for assessment of risk to human health or for preliminary trials of other potential plants suitable for phyto-management or phytoremediation, and for a wider range of contaminants. This will be a source of tremendously useful information which could provide a wide range of plant concentrations within which on-site plant concentration might fall, especially at sites with unknown spatial distribution of the contaminant. Results for both plant species provide very strong support for growing plants in different levels of heterogeneities in pot trials and in a field trial, as a more robust way of comparing the effectiveness or efficiency of the different plant species prior to on-site phytoremediation. Studies concerned with identification of suitable plant species for phytoremediation could take into consideration the spatial heterogeneity of contaminant in the field. This may be useful in making more effective selection of suitable plant species for specific contaminated sites.

This work also provides basis for studies on the effect of *in situ* heterogeneity of other contaminants in soil on root response either as individual study or a combination of contaminants in soil as these metals do not often exist in isolation in field scenario. The antagonistic and synergistic effects of such contaminants could be studied to improve the understanding of their geochemical pathway. Studies can also be expanded to essential trace elements or nutrients necessary for plant growth taking advantage of plant root response. This could be useful in increasing the uptake of such essential elements in food crops and vegetable plant species with the aim of improving human and animal dietary needs for essential microelements.

*B*rassica *napus* and *Brassica juncea* which showed species-specific differences in root response to heterogeneity. This could form a basis for future studies which could explore a wide range of plant species that could be used in building geochemical baseline data on plant root response (that incorporate metal heterogeneity) for many plant species, which could be tremendously useful in improving geochemical models of plant uptake for risk assessment, building predictive models of geochemical processes, phyto-management and phyto-remediation.

5. CONCLUSION

In conclusion, this study has shown that the spatial heterogeneity of Pb is a significant factor influencing plant growth and performance. It also showed that the differences in the root morphology could influence plant specific root response and behaviour to spatial heterogeneity, which could provide insights into varied plant Pb uptake and growth.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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